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(54) **Recombinant proteins of viruses associated with lymphadenopathy syndrome and/or acquired immune deficiency syndrome**

Rekombinante Proteine von mit Lymphadenopathiesyndrom und/oder erworbenem Immunschwächesyndrom assoziierten Viren

Protéines recombinantes de virus associé au syndrome lymhadenopatique et/ou syndrome d'immunodéficience acquise

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EP-A- 0 139 216
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EP-A- 0 185 444

- **SCIENCE, vol. 225, no. 4664, August 24, 1984 Washington DC J.A. LEVY et al. "Isolation of Lymphocytopathic Retro- viruses from San Francisco Patients with AIDS", pages 840-842**

EP 0 518 443 B1

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Description

Technical Field

[0001] This invention is in the field of lymphadenopathy syndrome and/or acquired immune deficiency syndrome.

Background Art

[0002] With the discovery of human T-cell lymphotropic virus-I (HTLV-I) as an infectious agent in humans, it was established that retroviruses could infect humans and could be the etiological agent of disease. After HTLV-I was established, a second retrovirus of the same family, HTLV-II was found in a hairy cell leukemia established strain. Since that time, other human retroviruses have been isolated which are associated with lymphadenopathy syndrome (LAS) and/or acquired immune deficiency syndrome (AIDS) victims. Various retroviruses have been isolated from individuals with AIDS (sometimes called HTLV-III) or LAS (sometimes called LAV). See for example, Barre-Sinoussi, et al, Science (1983) 220:868-871 and Montagnier, et al, Cold Spring Harbor Symposium (1984) in press; Vilmer, et al, Lancet (1984) 1:753, Popovic, et al, Science (1984) 224:497 and Gallo, et al, Science (1984) 224:500. A comparison of HTLV-III and LAV may be found in Feorino, et al, (1984), supra. See also, Klatzman, et al, Science (1984) 225: 59-62, Montagnier, et al, ibid (1984) 63-66, and the references cited therein for a survey of the field. A general discussion of the T-cell leukemia viruses may be found in Marx, Science (1984) 224:475-477. Levy, et al, Science (1984) 225:840-842 report the isolation of ARV (AIDS-associated retroviruses).

[0003] For the purposes of this application, these viruses (HTLV-III, LAV, and ARV) will be generically referred to as human T-cell lymphotropic retrovirus (hTLR). The hTLRs may be shown to be of the same class by being similar in their morphology, serology, reverse transcriptase optima and cytopathology, as identified in the above references. For example, the reverse transcriptase prefers Mg^{+2}, and has a pH optima of about 7.8.

Disclosure of the Invention

[0004] The invention is defined in the claims.

Brief Description of the Drawings

[0005]

Figure 1 is a restriction map of proviral DNA (ARV-2).

Figure 2 is the nucleotide sequence of ARV-2(9B). The amino acid sequences for the products of the gag, pol, and env genes are indicated. The U3, R, and U5 regions of the LTRs are also designated. The cap site is position +1. A 3 bp inverted repeat at the ends of the LTR, the TATA box at position -29, the sequence complementary to the 3'-end of the $tRNA^{lys}$ at position 183, and the polyadenylation signal at position 9174 are underlined. The overlines indicate the amino sequences determined from virion proteins. The nucleotides at the beginning of each line are numbered, and the amino acids at the end of each line are indicated.

Modes for Carrying Out the Invention

[0006] The hTLR DNA sequences are synthesized at least in part and may be used for expression of polypeptides which may be a precursor protein subject to further manipulation by cleavage, or a complete mature protein or fragment thereof. The smallest sequence of interest, so as to provide a sequence encoding an amino acid sequence capable of specific binding to a receptor, e.g., an immunoglobulin. will be 21 bp, usually at least 45 bp, exclusive of the initiation codon. The sequence may code for any greater portion of or the complete polypeptide. or may include flanking regions of a precursor polypeptide, so as to include portions of sequences or entire sequences coding for two or more different mature polypeptides. The sequence will usually be less than about 5 kbp, more usually less than about 3 kbp.

[0007] Sequences of particular interest having open reading frames (Figure 5) define the structural genes for the gag proteins (p16 and p25) and the env protein . It is to be understood that the above sequences may be spliced to other sequences present in the retrovirus, so that the 5'-end of the sequence may not code for the N-terminal amino acid of the expression product. The splice site may be at the 5'-terminus of the open reading frame or internal to the open reading frame. The initiation codon for the protein may not be the first codon for methionine, but may be the second or third methionine, so that employing the entire sequence indicated above may result in an extended protein. However, for the gag and env genes there will be proteolytic processing in mammalian cells, which processing may include the removal of extra amino acids.

[0008] In isolating the different domains the provirus may be digested with restriction endonucleases, the fragments electrophoresed and fragments having the proper size and duplexing with a probe, when available, are isolated, cloned in a cloning vector, and excised from the vector. The fragments may then be manipulated for expression. Superfluous nucleotides may be removed from one or both termini using Ba131 digestion. By restriction mapping convenient restriction sites may be located external or internal to the coding region. Primer repair or in vitro mutagenesis may be employed for defining a terminus, for insertions, deletion, point or multiple mutations, or the like, where codons may be changed, either cryptic or changing the amino acid. re-

striction sites introduced or removed, or the like. where the gene has been truncated, the lost nucleotides may be replaced using an adaptor. Adaptors are particularly useful for joining coding regions to ensure the proper reading frame.

[0009] The env domain of the hTLR genome can be obtained by digestion of the provirus with EcoRI and KpnI and purification of a 3300 base pair (bp) fragment, which fragment contains about 400 bp of 5' non-coding and about 200 bp of 3' non-coding region. Three different methionines coded for by the sequence in the 5' end of the open reading frame may serve as translational initiation sites.

[0010] Digestion of proviral sequences with SacI and EcoRV provides a fragment of about 2300 bp which contains the gag domain and a second small open reading frame towards the 3' end of the gag region. The gag domain is about 1500 bp and codes for a large precursor protein which is processed to yield proteins of about 25.000 (p25), 16.000 (p16) and 12,000 (p12) daltons. Digestion with SacI and BglII may also be used to obtain exclusively the gag domain with p12, p25 and partial p16 regions.

[0011] The hTLR DNA sequences may be labeled with isotopic or non-isotopic labels or markers and be used as DNA probes to detect the presence of native hTLR nucleotide sequences in samples suspected of containing same.

[0012] The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

1. AIDS related virus-2 (ARV-2) purification and preparation of viral RNA.

[0013] HUT-78 cells infected with ARV-2 (ATCC Accession No. CRL B597, deposited on August 7, 1984) were obtained from Dr. Jay Levy, University of California, San Francisco. Cultures were grown for two weeks in RPM1 medium with 10% fetal calf serum. Cultures were centrifuged at 2 Krpm for 1 hr at 4°C using a SW-2B rotor. The pellet, containing the virus. was resuspended in 10 mM Tris-HCl, pH 7.5 on ice. The resuspended pellet was treated with 10 µg of DNase (Boehringer Mannhein) and was layered onto a linear sucrose gradient (15-50% in 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 20 mM NaCl). The gradient was spun at 34 Krpm for 4 hr at 4°C, in SW-41 rotor. Five 2.5 ml fractions were collected and an aliquot of each was electrophoresed in a 1% agarose, 5 mM methyl mercury hydroxide gel (Bailey and Davidson. Anal Biochem (1976) 70:75-85) to determine which contained the 9 kb viral RNA. The fraction containing the viral RNA was diluted: to 10 ml in 10 mM Tris-HCl, pH 7.5, 1 mM EDTA and was centrifuged at 34 Krpm for 2 hr at 4°C. The pellet was resuspended in 20 mM Tris-HCl, pH 7.6, 10 mM EDTA, 0.1% SDS, and 200 µg/ml proteinase K. Incubation was carried out for 15 min at room temperature. The mixture was extracted with phenol and the aqueous phase was made 400 mM NaCl and precipitated with ethanol. The pellet was resuspended in water and stored at -70°C.

[0014] To purify the viral RNA from the nucleic acid pellet obtained as described above, a sample was electrophoresed in a low-melting 1% agarose gel containing 5 mM Methyl mercury hydroxide. After electrophoresis, the gel was stained with 0.1% ethidium bromide and nucleic acid bands were visualized under UV light. The region corresponding to 9 kb was cut from the gel and the agarose was melted at 70°C for 2 to 3 min in three volumes of 0.3 M NaCl, 10 mM Tris, pH 7.5, 1 mM EDTA. The mixture was extracted with an equal volume of phenol. The aqueous phase was reextracted with phenol and was precipitated with ethanol. The pellet was washed with cold 95% ethanol, air dried, resuspended in water and stored at -70°C until use. One hundred ml of culture medium yielded 0.5 to 1 µg of purified RNA.

2. Synthesis of labeled homologous viral probe.

[0015] A ^{32}P-labeled cDNA was made to the gel purified viral RNA using random primers (calf thymus primers) prepared as described in Maniatis, et al, A Laboratory Manual, Cold Spring Harbor, NY, 1982. The reaction mixture contained 2 µl of 0.5 M $MgCl_2$; 5 µl of 0.1 M dithiothreitol; 2.5 µl each of 10 mM dATP, 10 mM dGTP and 10 mM dTTP; 2.5 µl calf thymus primer ($100A_{260}$/ml); 0.5 µg viral RNA: 5 µl of actinomycin D (200 µg/ml); 10 µl of ^{32}P-dCTP (> 3000 Ci/mmole, 1 mCi/ml) and 1 µl of AMV reverse transcriptase (17 units/µl) in a 50 µl reaction volume. The reaction was incubated for 1 hr at 37°C. The probe was purified away from free nucleotides by gel filtration using a Sephadex G50 column. The void volume was pooled, NaCl was added to a final concentration of 400 mM and carrier single-stranded DNA to 100 µg/ml, and the cDNA was precipitated with ethanol. The pellet was resuspended in water and incorporated ^{32}P counts were determined.

3. Detection of ARV sequences in polyA+ RNA prepared from infected HUT-78 cells.

[0016] PolyA+ RNA was prepared from HUT-78 cells infected with ARV-2, ARV-3 or ARV-4 (three different isolates from three different AIDS patients) and from uninfected HUT-78 cells. The polyA, RNA was electrophoresed on 1% agarose gels containing 5 mM methyl mercury hydroxide (Bailey and Davidson, supra), was transferred to nitrocellulose filters, and hybridized with the homologous probe prepared as described in Section 2. Hybridizations were carried out in 50% formamide, 3 x SSC at 42°C. Washes were at 50°C in 0.2 x SSC. A 9 kbp band was present in all three samples of infected HUT-7B cells. This band was absent in polyA+ from uninfected cells.

4. Detection of ARV sequences in infected and non-infected HUT-78 cells.

[0017] High molecular weight DNA (chromosomal) was prepared from cultures of HUT-78 cells infected with ARV-2 and from non-infected HUT-78 cells following the procedure of Luciw, et al, Molec and Cell Biol (1984) 4:1260-1269. The DNA was digested with restriction enzyme(s), electrophoresed in 1% agarose gels and blotted onto nitrocellulose following the procedure described by Southern, (1975), supra. Blots were hybridized with the ^{32}P-labeled probe (10^6 cpm/blot) in a mixture containing 50% formamide, 3 x SSC, 10 mM Hepes, pH 7.0, 100 µg/ml denatured carrier DNA, 100 µg/ml yeast RNA and 1 x Denhardt's for 36 hr at 42°C. Filters were washed once at room temperature in 2 x SSC and twice at 42°C in 0.2 x SSC, 0.1% SDS. Filters were air dried and exposed to X-Omat film using an intensifying screen.

[0018] The homologous ^{32}P-probe to ARV-2 hybridized specifically to two bands in the DNA from infected cells restricted with SacI. These bands were absent when DNA of non-infected cells was used, indicating that the probe is hybridizing specifically to infected cells presumably to the provirus integrated in the chromosomal DNA. The molecular weight of the bands is approximately 5 kb and 3 kb.

[0019] In order to determine if different enzymes would cut the proviral sequence, several other restriction digestions of the cell DNA were carried out using EcoRI, SphI or KpnI or double digestions using two of them. Southern results show specific bands hybridizing when DNA of infected cells is used. Figure 1 shows a schematic map of the positions of restriction enzyme sites in the proviral sequence, and indicates fragment sites.

5. Cloning of proviral ARV-2 DNA.

[0020] High molecular weight cell DNA from infected HUT-78 cells was prepared following the procedure of Luciw, et al, supra. The DNA was digested with EcoRI, which cuts once in the previrus, centrifuged in a sucrose gradient and fractions corresponding to 8-15 kb were pooled, dialyzed and concentrated by ethanol precipitation. The bacteriophage λ derivative cloning vector, EMBL-4 (Karn, et al, Methods Enzymol (1983) 101:3-19) was digested to completion with a mixture of EcoRI, BamHI and SalI restriction enzymes and the DNA then deproteinized by phenol-chloroform extraction, precipitated with cold ethanol and resuspended in ligation buffer. The EMBL-4 phage DNA and EcoRI digest of cellular DNA were mixed and ligated and the resultant recombinant phage genomes packaged in vitro. After phage infection of λ-sensitive E. coli (DP50supF), about 500,000 phage plaques were transferred onto nitrocellulose filters, DNA was fixed and the filters were screened with a homologous ^{32}P-probe prepared as described in Section 2. Eleven recombinant phage out of 500,000 phage annealed in the initial double-lift screening method (Maniatis, et al, Molecular Cloning, A Laboratory Manual, NY, 1982) to viral cDNA probe, and these were further plaque-purified and propagated in large liquid cultures for preparation of recombinant DNA. Plaque-purified phage containing ARV DNA were propagated in liquid culture in E. coli DP50supF; phage particles were harvested and banded in CsCl gradients and recombinant phage DNA was prepared by phenol extraction followed by ethanol precipitation (Maniatis, et al, supra). One µg of purified phage DNA was digested with restriction enzymes, electrophoresed on 1% agarose gels, and visualized with ethidium bromide under ultraviolet light. The DNA from these gels was transferred to nitrocellulose and annealed with viral cDNA probe.

[0021] One of the 11 phage, designated λ ARV-2(9B). was deposited at the ATCC on 25 January 1985 and given Accession No. 40158. λ ARV-2(9B) contained an insertion of full-length proviral DNA along with flanking cell sequences. Digestion of λ ARV-2(9B) DNA with SacI yielded viral DNA fragments of 3.8 kb and 5.7 kb. EcoRI digestion of λ ARV-2(9B) produced virus containing DNA species at 6.4 kb and 8.0 kb; a double digest of SacI and EcoRI gave viral DNA fragments at 3.8 kb and 5.4 kb. This pattern is consistent with that of a provirus linked to cell DNA.

[0022] In addition to λ ARV-2(9B). phage was obtained that (1) possessed the left half of the viral genome from the EcoRI site in viral DNA extending into flanking cell DNA (λARV-2(8A)) and (2) phage that had the right half of the viral genome (λARV-2(7D)) from the EcoRI site in viral DNA extending into flanking cell DNA. Bacteriophages λARV-2(7D) (right) and λARV-2(8A) (left) were deposited at the ATCC on October 26, 1984 and given Accession Nos. 40143 and 40144, respectively.

6. Polymorphism.

[0023] To measure the relatedness of independent ARV isolates, restriction enzyme digests of DNA from HUT-78 cells infected with ARV-3 and ARV-4 were analyzed with the probe made from cloned ARV-2 DNA. The SacI digest of ARV-3 DNA was similar to that of ARV-2 whereas the HindIII digests displayed different patterns. The SacI digest and the PstI digest of ARV-4 DNA differed from the corresponding digests of ARV-2 DNA. The intensity of the annealing signals obtained with ARV-3 and ARV-4 samples was much lower (about 10-fold less) than that for ARV-2 DNA probably as a result of the fact that fewer cells were infected in the ARV-3 and ARV-4 cultures. The viral-specific DNA fragments produced by SacI treatment of ARV-3 and ARV-4 DNA totaled 9.0-9.5 kbp, a value similar to that of ARV-2 and in consonance with the RNA genome sizes.

7. Sequencing of proviral DNA.

[0024] Fragments or subfragments of ARV-2 DNA from λ phage 9B were prepared and cloned into M13 according to conventional procedures (Maniatis, et al, supra). Sequencing was performed according to Sanger, et al, Proc Natl Acad Sci USA (1977) 74:5463, using the universal M13 primer or chemically synthesized primers complementary to ARV-2 sequence. The sequence is shown in Figure 2.

Claims

1. A process for the preparation of an isolated DNA polynucleotide comprising a fragment of at least 21 bp from the gag or env region of the ARV-2 sequence of Figure 2, wherein the DNA polynucleotide is chemically synthesised at least in part.

2. The process of claim 1, wherein the DNA polynucleotide comprises a fragment of at least 21 bp from the gag region of the ARV-2 sequence of Figure 2.

3. The process of claim 1, wherein the DNA polynucleotide comprises a fragment of at least 21 bp from the env region of the ARV-2 sequence of Figure 2.

4. The process of any preceding claim, wherein the DNA polynucleotide is labelled.

5. The process of claim 4, wherein the DNA polynucleotide is labelled with an isotopic label.

6. The process of claim 4, wherein the DNA polynucleotide is labelled with a non-isotopic label.

Patentansprüche

1. Verfahren zur Herstellung eines isolierten DNA-Polynucleotids, das ein Fragment von mindestens 21 bp aus der gag-Region oder der env-Region der ARV-2-Sequenz gemäß Figur 2 umfaßt, wobei das DNA-Polynucleotid mindestens teilweise chemisch synthetisiert wird.

2. Verfahren nach Anspruch 1, wobei das DNA-Polynucleotid ein Fragment von mindestens 21 bp aus der gag-Region der ARV-2-Sequenz gemäß Figur 2 umfaßt.

3. Verfahren nach Anspruch 1, wobei das DNA-Polynucleotid ein Fragment von mindestens 21 bp aus der env-Region der ARV-2-Sequenz gemäß Figur 2 umfaßt.

4. Verfahren nach einem der vorhergehenden Ansprüche, wobei das DNA-Polynucleotid markiert ist.

5. Verfahren nach Anspruch 4, wobei das DNA-Polynucleotid mit einem isotopischen Marker markiert ist.

6. Verfahren nach Anspruch 4, wobei das DNA-Polynucleotid mit einem nicht-isotopischen Marker markiert ist.

Revendications

1. Procédé pour la préparation d'un polynucléotide d'ADN isolé comprenant un fragment d'au moins 21 pb de la région gag ou env de la séquence ARV-2 de la figure 2, dans lequel le polynucléotide d'ADN est chimiquement synthétisé au moins en partie.

2. Procédé selon la revendication 1, dans lequel le polynucléotide d'ADN comprend un fragment d'au moins 21 pb de la région gag de la séquence ARV-2 de la figure 2.

3. Procédé selon la revendication 1, dans lequel le polynucléotide d'ADN comprend un fragment d'au moins 21 pb de la région env de la séquence ARV-2 de la figure 2.

4. Procédé selon l'une quelconque des revendications précédentes, dans lequel le polynucléotide d'ADN est marqué.

5. Procédé selon la revendication 4, dans lequel le polynucléotide d'ADN est marqué avec un marqueur isotopique.

6. Procédé selon la revendication 4, dans lequel le polynucléotide d'ADN est marqué avec un marqueur non isotopique.

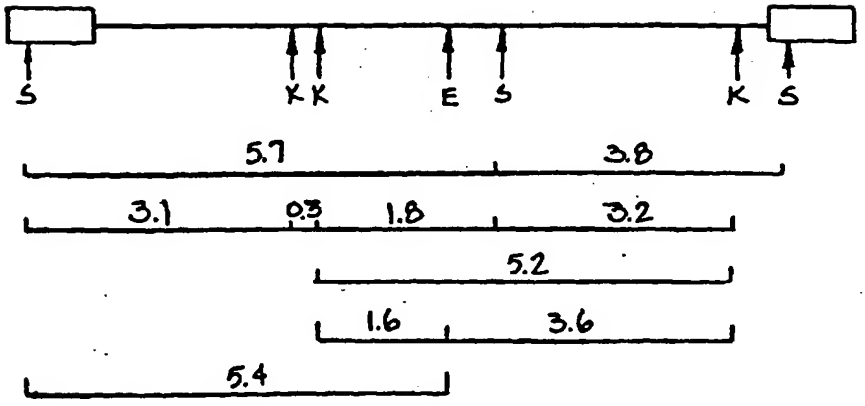


FIG. 1

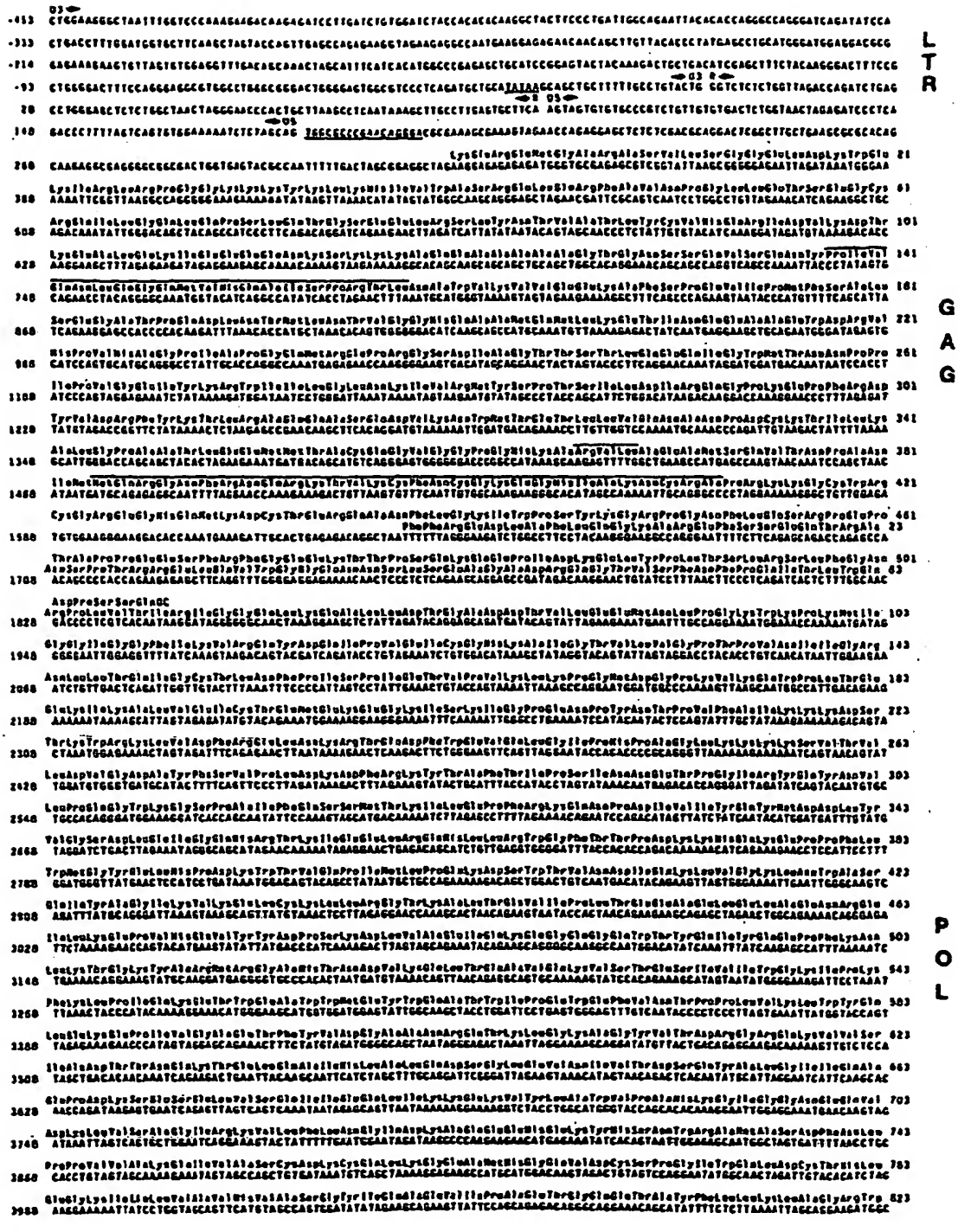


FIG. 2-1

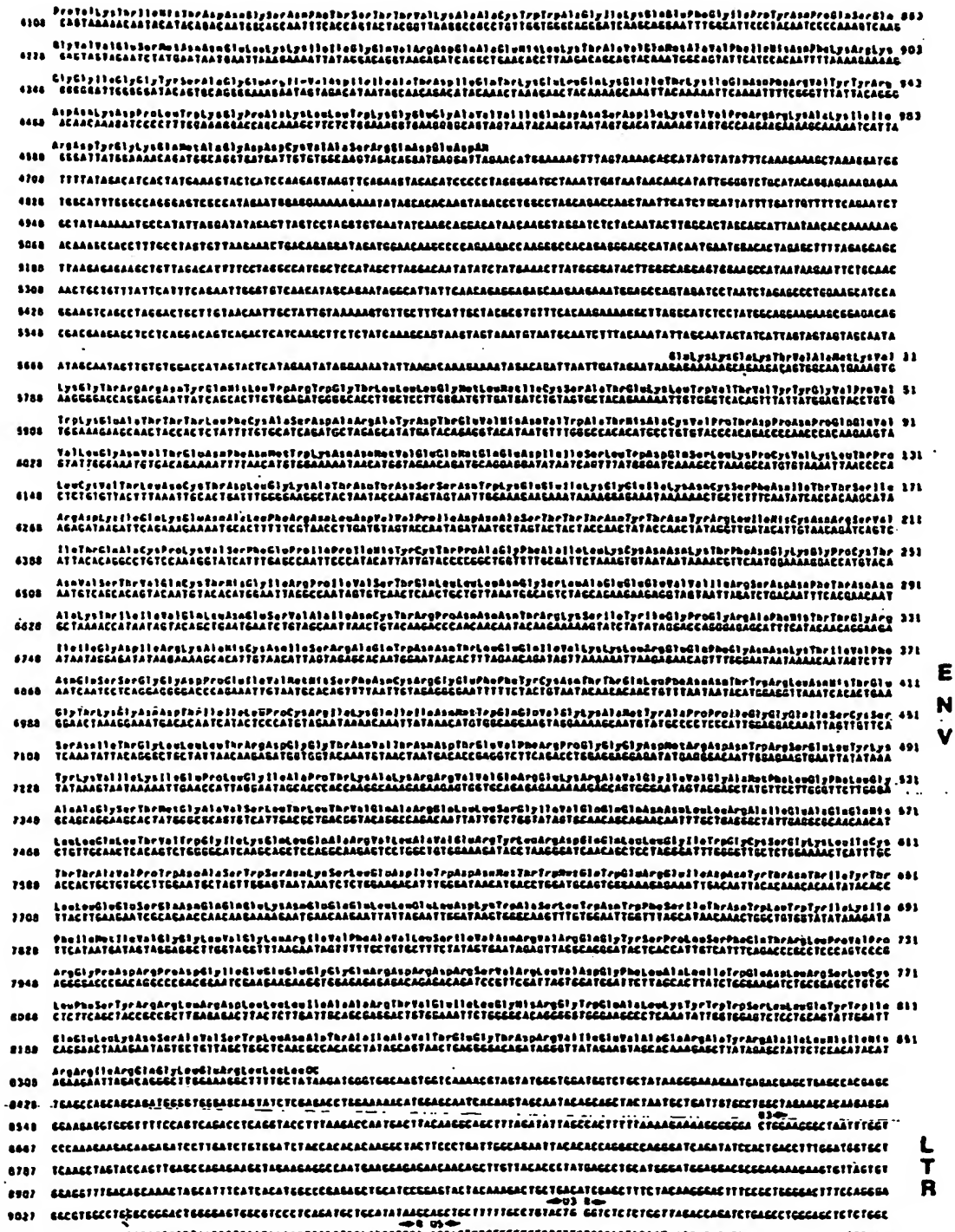


FIG. 2-2

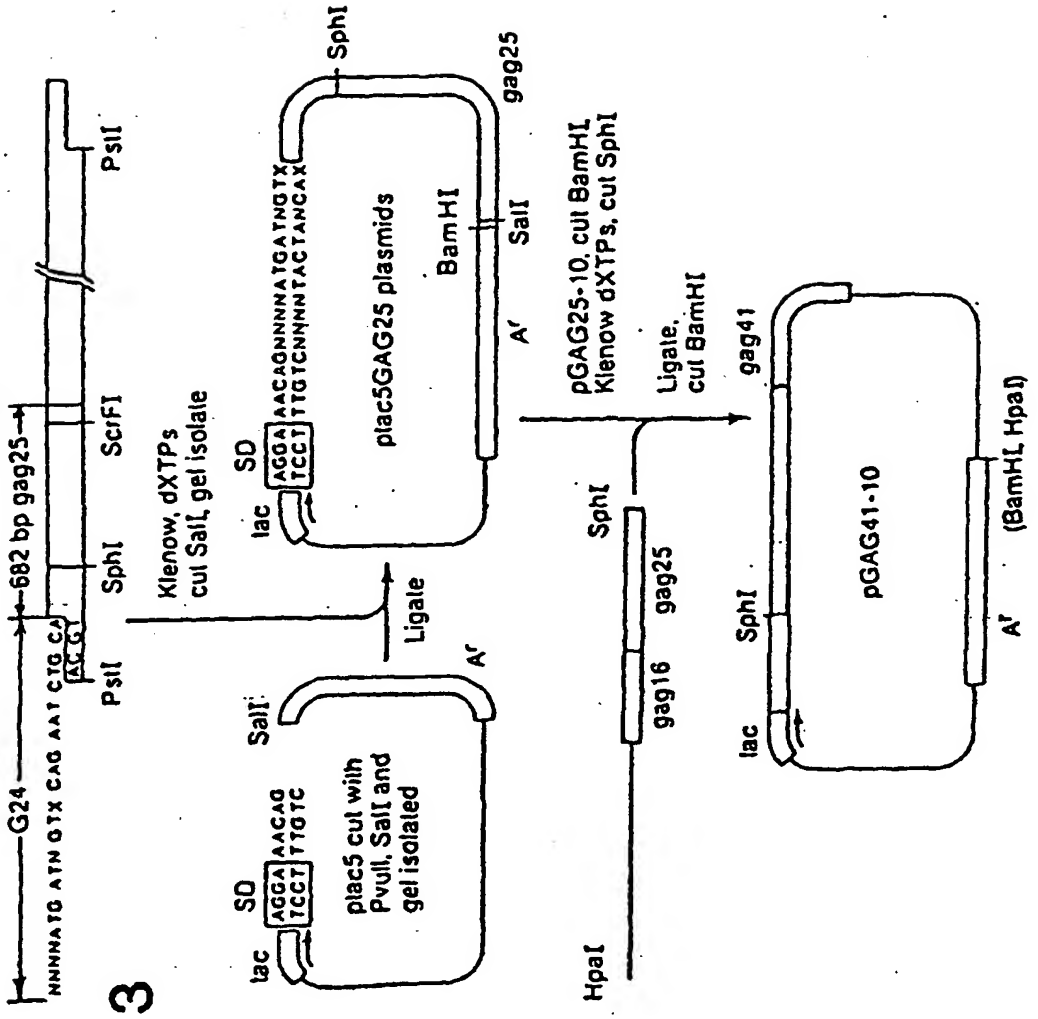
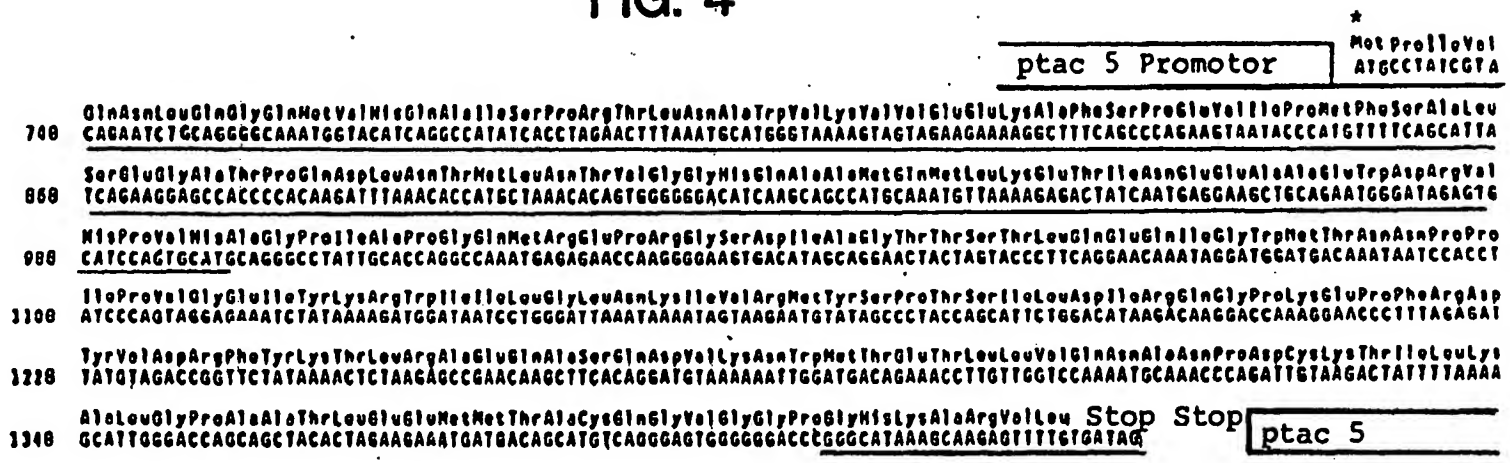

SalI
682 bp gag25
G24
NNNNATG ATN GTX CAG AAT CTG CA
AC GT
PstI
SphI
ScrFI
PstI
Klenow, dXTPs
cut SalI, gel isolate
lac
SD
AGGA
TCCT
AACAONNNNATGATNGTX
TTGTCNNNNTACTANCAX
plac5GAG25 plasmids
SphI
gag25
BamHI
SalI
A'
Ligate
lac
SD
AGGA
TCCT
AACAG
TTGTC
plac5 cut with
PvuII, SalI and
gel isolated
SalI
A'
pGAG25-10, cut BamHI,
Klenow dXTPs, cut SphI
Ligate,
cut BamHI
gag41
SphI
gag25
gag16
HpaI
lac
SphI
pGAG41-10
A'
(BamHI, HpaI)

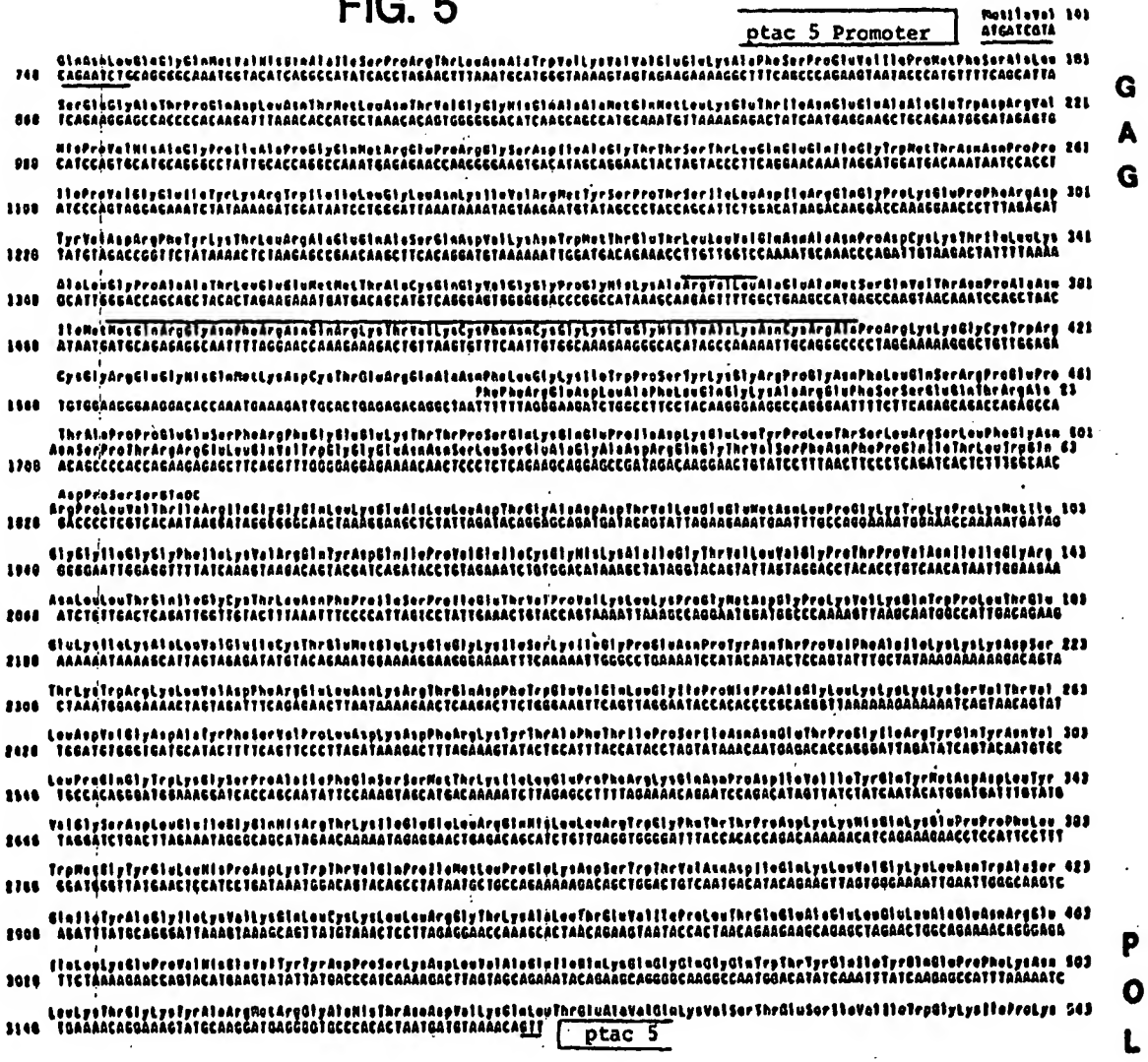
FIG. 3

FIG. 4



EP 0 518 443 B1

FIG. 5



EP 0 518 443 B1

FIG. 6

ARV GAG p16 - synthetic Parts A and B

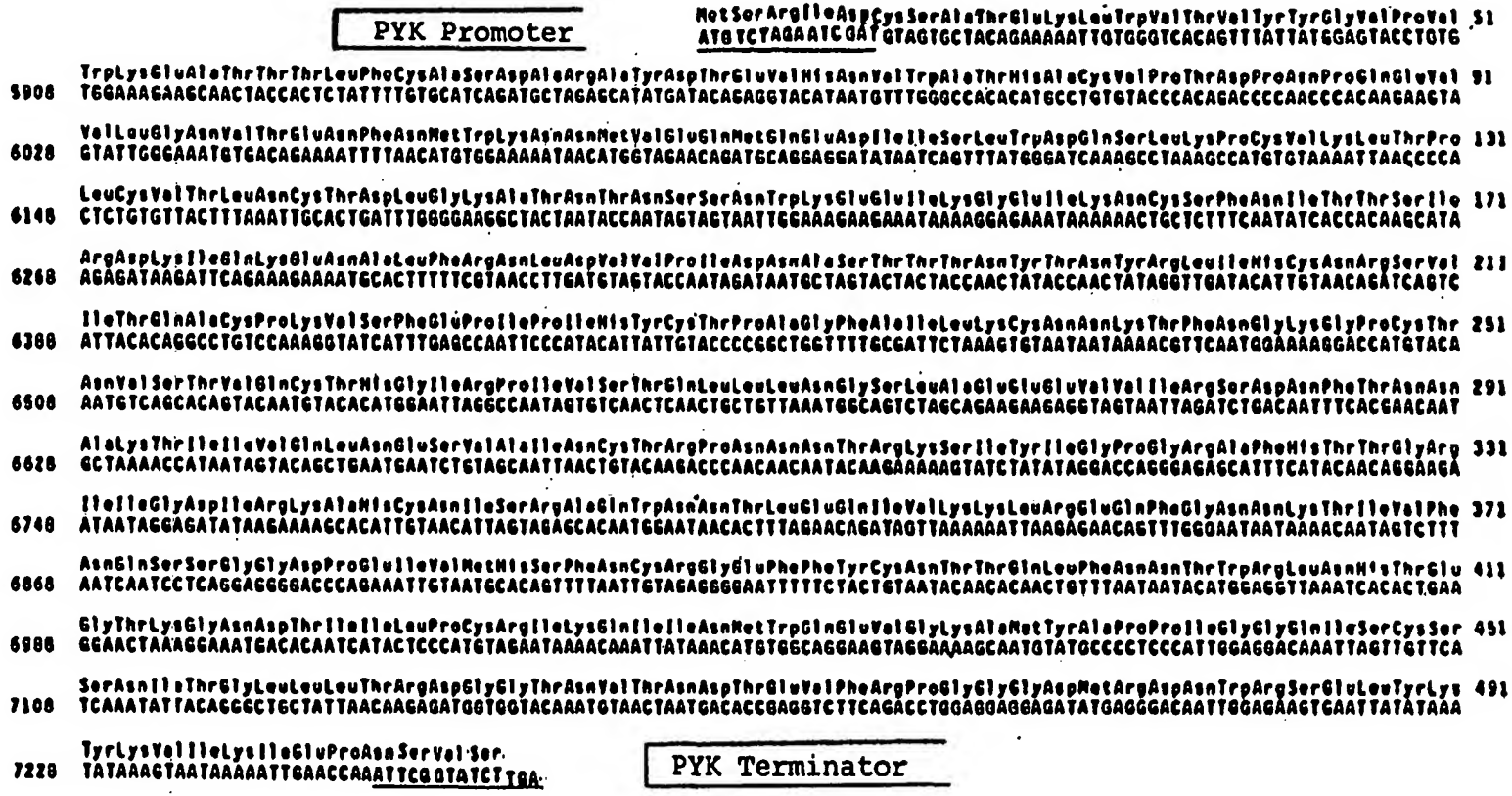
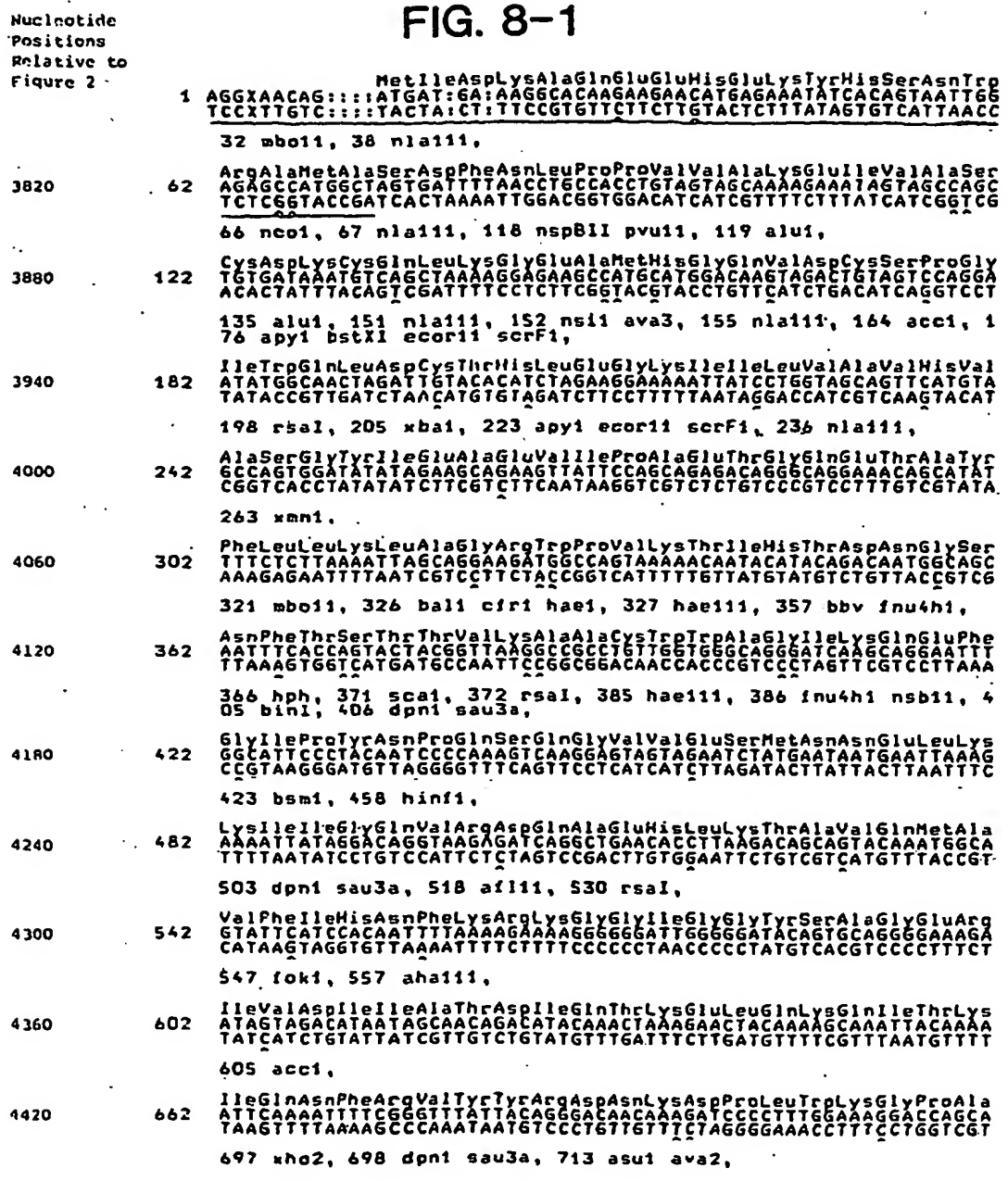


FIG. 7

EP 0 518 443 B1

FIG. 8-1


Nucleotide Positions Relative to Figure 2
MetIleAspLysAlaGlnGluGluHisGluLysTyrHisSerAsnTrp
1 AGGXAACAG::::ATGAT:GA:AAGGCACAAGAAGAACATGAGAAATATCACAGTAATTGG
TCCXTTGTC::::TACTA:CT:TTCCGTGTTCTTCTTGTACTCTTTATAGTGTCATTAACC
32 mbo11, 38 nla111,
ArgAlaMetAlaSerAspPheAsnLeuProProValValAlaLysGluIleValAlaSer
3820 62 AGAGCCATGGCTAGTGATTTTAACCTGCCACCTGTAGTAGCAAAAGAAATAGTAGCCAGC
TCTCGGTACCGATCACTAAAATTGGACGGTGGACATCATCGTTTTCTTTATCATCGGTCG
66 nco1, 67 nla111, 118 nspBII pvu11, 119 alu1,
CysAspLysCysGlnLeuLysGlyGluAlaMetHisGlyGlnValAspCysSerProGly
3880 122 TGTGATAAATGTCAGCTAAAAGGAGAAGCCATGCATGGACAAGTAGACTGTAGTCCAGGA
ACACTATTTACAGTCGATTTTCCTCTTCGGTACGTACCTGTTCATCTGACATCAGGTCCT
135 alu1, 151 nla111, 152 nsi1 ava3, 155 nla111, 164 acc1, 1
76 apy1 bstXI ecor11 scrF1,
IleTrpGlnLeuAspCysThrHisLeuGluGlyLysIleIleLeuValAlaValHisVal
3940 182 ATATGGCAACTAGATTGTACACATCTAGAAGGAAAAATTATCCTGGTAGCAGTTCATGTA
TATACCGTTGATCTAACATGTGTAGATCTTCCTTTTTAATAGGACCATCGTCAAGTACAT
198 rsa1, 205 xba1, 223 apy1 ecor11 scrF1, 236 nla111,
AlaSerGlyTyrIleGluAlaGluValIleProAlaGluThrGlyGlnGluThrAlaTyr
4000 242 GCCAGTGGATATATAGAAGCAGAAGTTATTCCAGCAGAGACAGGGCAGGAAACAGCATAT
CGGTCACCTATATATCTTCGTCTTCAATAAGGTCGTCTCTGTCCCGTCCTTTGTCGTATA
263 xmn1,
PheLeuLeuLysLeuAlaGlyArgTrpProValLysThrIleHisThrAspAsnGlySer
4060 302 TTTCTCTTAAAATTAGCAGGAAGATGGCCAGTAAAAACAATACATACAGACAATGGCAGC
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321 mbo11, 326 ball cfr1 hae1, 327 hae111, 357 bbv fnu4h1,
AsnPheThrSerThrThrValLysAlaAlaCysTrpTrpAlaGlyIleLysGlnGluPhe
4120 362 AATTTCACCAGTACTACGGTTAAGGCCGCCTGTTGGTGGGCAGGGATCAAGCAGGAATTT
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366 hph, 371 sca1, 372 rsa1, 385 hae111, 386 fnu4h1 nsb11, 4
05 bin1, 406 dpn1 sau3a,
GlyIleProTyrAsnProGlnSerGlnGlyValValGluSerMetAsnAsnGluLeuLys
4180 422 GGCATTCCCTACAATCCCCAAAGTCAAGGAGTAGTAGAATCTATGAATAATGAATTAAAG
CCGTAAGGGATGTTAGGGGTTTCAGTTCCTCATCATCTTAGATACTTATTACTTAATTTC
423 bsm1, 458 hinf1,
LysIleIleGlyGlnValArgAspGlnAlaGluHisLeuLysThrAlaValGlnMetAla
4240 482 AAAATTATAGGACAGGTAAGAGATCAGGCTGAACACCTTAAGACAGCAGTACAAATGGCA
TTTTAATATCCTGTCCATTCTCTAGTCCGACTTGTGGAATTCTGTCGTCATGTTTACCGT
503 dpn1 sau3a, 518 afl11, 530 rsa1,
ValPheIleHisAsnPheLysArgLysGlyGlyIleGlyGlyTyrSerAlaGlyGluArg
4300 542 GTATTCATCCACAATTTTAAAAGAAAAGGGGGGATTGGGGGATACAGTGCAGGGGAAAGA
CATAAGTAGGTGTTAAAAATTTTCTTTTCCCCCCTAACCCCCTATGTCACGTCCCCTTTCT
547 fok1, 557 aha111,
IleValAspIleIleAlaThrAspIleGlnThrLysGluLeuGlnLysGlnIleThrLys
4360 602 ATAGTAGACATAATAGCAACAGACATACAAACTAAAGAACTACAAAAGCAAATTACAAAA
TATCATCTGTATTATCGTTGTCTGTATGTTTGATTTCTTGATGTTTTCGTTTAATGTTTT
605 acc1,
IleGlnAsnPheArgValTyrTyrArgAspAsnLysAspProLeuTrpLysGlyProAla
4420 662 ATTCAAAATTTTCGGGTTTATTACAGGGACAACAAAGATCCCCTTTGGAAAGGACCAGCA
TAAGTTTTAAAAGCCCAAATAATGTCCCTGTTGTTTCTAGGGGAAACCTTTCCTGGTCGT
697 xho2, 698 dpn1 sau3a, 713 asu1 ava2,

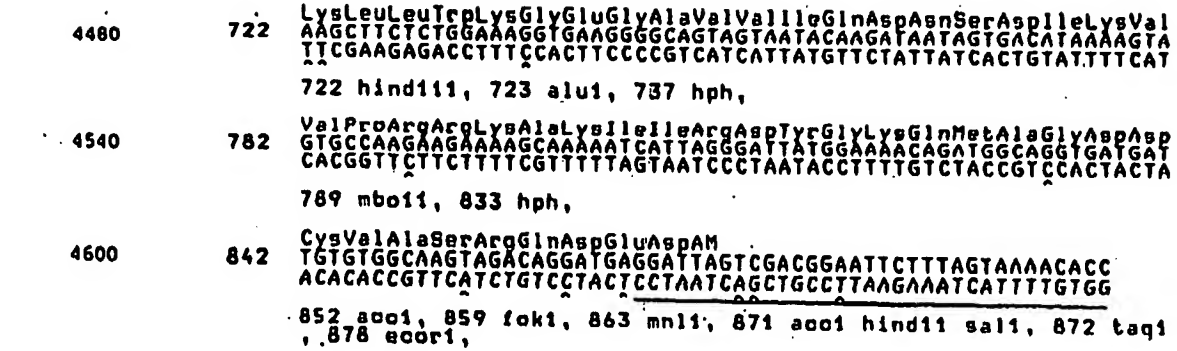
4480
722
LysLeuLeuTrpLysGlyGluGlyAlaValValIleGlnAspAsnSerAspIleLysVal
AAGCTTCTCTGGAAAGGTGAAGGGGCAGTAGTAATACAAGATAATAGTGACATAAAAGTA
TTCGAAGAGACCTTTCCACTTCCCCGTCATCATTATGTTCTATTATCACTGTATTTTCAT
722 hind111, 723 alu1, 737 hph,
4540
782
ValProArgArgLysAlaLysIleIleArgAspTyrGlyLysGlnMetAlaGlyAspAsp
GTGCCAAGAAGAAAAGCAAAAATCATTAGGGATTATGGAAAACAGATGGCAGGTGATGAT
CACGGTTCTTCTTTTCGTTTTTAGTAATCCCTAATACCTTTTGTCTACCGTCCACTACTA
789 mbo11, 833 hph,
4600
842
CysValAlaSerArgGlnAspGluAspAM
TGTGTGGCAAGTAGACAGGATGAGGATTAGTCGACGGAATTCTTTAGTAAAACACC
ACACACCGTTCATCTGTCCTACTCCTAATCAGCTGCCTTAAGAAATCATTTTGTGG
852 acc1, 859 fok1, 863 mnl1, 871 acc1 hind11 sal1, 872 taq1
, 878 ecor1,

FIG. 8-2